

Amendments to the Specification:

Please replace the Abstract with the following Abstract:

The invention provides processes for ~~A process of~~ generating transgenic plants or plant cells transformed on their plastome, comprising introducing into plant plastids a first ~~DNA molecule~~ and a second DNA molecule, wherein ~~said~~ the first DNA molecule contains a first region homologous to a region of the plastome for directing plastome integration and a first sequence of interest, and ~~said~~ the second DNA molecule contains a second region homologous to a region of the plastome ~~for directing plastome integration~~ and a second sequence of interest, whereby a sequence segment of ~~said~~ the first sequence of interest is homologous to a sequence segment of ~~said~~ the second sequence of interest, and selecting transformants having an integration sequence stably integrated in the plastome, whereby ~~said~~ the integration sequence contains at least a portion of ~~said~~ the first and ~~at least a portion of said~~ second sequences of interest as a continuous sequence. The invention further provides plant cells, plants, and seeds of plants produced by such processes.

Please replace the paragraph that bridges pages 28 and 29 of the application with the following paragraph:

Plastid transformants (cycle-0) were identified by PCR using total DNA isolated with the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). To determine the presence of the *aadA* gene the primers oSH81 (SEQ ID NO:3) (5'-CTATCAGAGGTAGTTGGCGTC-3') and oFCH60 (SEQ ID NO:1) (5'-CACTACATTTCGCTCATCGCC-3') were used. The PCR program was as follows: 3 min at 94 °C, 1 cycle; 45 sec at 94 °C, 45 sec at 55 °C, 2 min at 72 °C, 30 cycles; final extension at 72 °C for 10 min. The results showed that 48 lines from 54 analysed (6 bombarded leaves) gave the expected amplification product of 504 bp. To prove correct integration of the *aadA* cassette within the tobacco plastome primers oSH58 (SEQ ID NO:2) (5'-TATTCCGACTTCCCCAGAGC-3') and oFCH60 (SEQ ID NO:1) (5'-CACTACATTTCGCTCATCGCC-3') were used. Primer oSH58 is located outside (downstream) of the right flank of pKCZ in the tobacco plastome and in combination with

oFCH60 can only give the expected product of 2106 bp upon integration of the *aadA* expression cassette between *trnR* and *trnN* in the inverted repeat. The PCR program was as follows: 5 min at 94 °C, 1 cycle; 45 sec at 94 °C, 45 sec at 55 °C, 3.5 min at 72 °C, 35 cycles; final extension at 72 °C for 7 min. All 48 of the *aadA* PCR positive lines showed the expected right-flank-*aadA* product of 2106 bp.

Please replace the paragraph that bridges pages 29 and 30 with the following paragraph:

Normally, the production of stable plastid transformants is thought to occur via two simultaneous recombination events occurring between the left and right flanks of the transforming molecule and the plastome (as depicted in figure 1). An alternative mechanism is presented in figure 2. Here, complete integration of the vector occurs first, via recombination with one flank only (either left or right) with the plastome, resulting in the generation of a hypothetical unstable intermediate. Subsequent additional recombination events can then take place between the duplicated flanks in this molecule to generate either the wild-type situation or a stably integrated *aadA* cassette. In order to test for this possibility, PCR was performed using primers oSH3 (SEQ ID NO:4) (5'-GGCATCAGAGCAGATTG-3') and oSH58 (SEQ ID NO:2) (5'- TATTCCGACTTCCCCAGAGC-3'). Primer oSH3 is located within the vector backbone of pKCZ (pUC18) and primer oSH58 is located outside (downstream) of the right flank of pKCZ in the tobacco plastome. A product of 2638 bp can only be obtained with these two primers when complete pKCZ integration has occurred as shown in figure 2. No PCR product of the expected size will be obtained from the wild type plastome fragment (comprising left and right flanks) since the binding site for oSH3 is absent. The PCR program was as follows: 5 min at 94 °C, 1 cycle; 45 sec at 94 °C, 45 sec at 55 °C, 3.5 min at 72 °C, 35 cycles; final extension at 72 °C for 7 min. Nine of the 10 cycle-0 transformants analysed showed a PCR product of 2.6 kb which would be consistent with complete integration of pKCZ into the plastid genome within these lines (fig. 3A). No product of the correct size was observed in the wild type control or in sample 1:1. Since complete integration of pKCZ results in the formation of an unstable intermediate it is to be expected that with increasing time additional recombination events between the duplicated flanks in

this molecule will lead to either the wild-type situation or a stably integrated *aadA* cassette. As such DNA samples prepared from cycle-I and cycle-II plant material were analysed by PCR with primers oSH3 and oSH58. If the model presented in fig. 2 is correct the probability of amplifying the 2638 bp band with primers oSH3 and oSH58 should be reduced with each regeneration cycle on selection. The results suggest that this is indeed the case since only 5 of the 10 cycle-I lines analysed gave a strong PCR product of the expected size (fig. 3B). Furthermore, in cycle-II the number of lines showing clear amplification of the expected 2638 bp band was further reduced.

Please replace the paragraph that begins on page 30 at line 11 with the following paragraph:

The model presented in figure 2 also predicts that all cycle-II lines which are negative for complete vector integration should still show PCR signals consistent with a stably integrated *aadA* cassette due to the molecular rearrangements previously described. To prove integration of the *aadA* cassette within the tobacco plastome primers oSH58 (SEQ ID NO: 2) (5'-TATTCCGACTTCCCCAGAGC-3') and oFCH60 (SEQ ID NO: 1) (5'-CACTACATTTGCTCATCGCC-3') were used. The PCR program was as follows: 5 min at 94 °C, 1 cycle; 45 sec at 94 °C, 45 sec at 55 °C, 3.5 min at 72 °C, 35 cycles; final extension at 72 °C for 7 min. All 10 of the cycle-II transformants show the expected right-flank-*aadA* product of 2106 bp (fig. [[3D]] 3E) which would be consistent with the scenario shown in figure 2.

Please replace the paragraph that bridges pages 30 and 31 with the following paragraph:

The right flanking region was amplified from tobacco plastid DNA (bp 132279 to bp 133390 of the *N. tabacum* plastome) with modifying primers 5'-TGGAGCTCGAATTGCCGCGAGCAAAGATATTAATG -3' (SEQ ID NO: 5) and 5'-TACGAATTCAAGAGAAGGTCACGGCGAGAC-3' (SEQ ID NO: 6), introducing an *SacI* recognition

site at the 5'-end and an EcoRI recognition site at the 3'-end. The PCR product was purified and digested with SacI and EcoRI and ligated into a pUC18 plasmid which was digested with the same enzymes. The rpl32 promoter was amplified from tobacco plastid DNA (bp 113917 to bp 114055 of the *N. tabacum* plastome) with modifying primers 5'-GACCCTGCAGGCAAAAAATCTCAAATAGCC -3' (SEQ ID NO: 7) and 5'-CGGGATCCGATTTTTCTTTAGACTTCGG-3' (SEQ ID NO: 8), introducing a PstI recognition site at the 5'-end and a BamHI recognition site at the 3'-end. The PCR product was reamplified with modifying primers 5'-CGGGATCCGATTTTTCTTTAGACTTCGG-3' (SEQ ID NO: 9) and 5'-CGAGCTCCACCGCGGTGGCGGCCGTCGACCCTGCAGGCAAAAAATCTC-3' (SEQ ID NO: 10) to introduce a new multi cloning site containing a SacI recognition site at the 5'-end. The resulting PCR product was digested with BamHI and SacI and ligated into the similar restricted pUC18 vector containing the right flanking region. The psbA-5'-UTR was amplified from tobacco plastid DNA (complementary to bp 1598 - bp 1680 of the *N. tabacum* plastome) with modifying primers 5'-CGGGATCCAAAAAGCCTTCCATTTTCTATTT-3' (SEQ ID NO: 11) and 5'-TTGCAGCCATGGTAAAATCTTGGTTTATT-3' (SEQ ID NO: 12) introducing a BamHI recognition site at the 5'-end and a NcoI recognition site at the 3'-end. The PCR product was digested with NcoI and BamHI. The aadA sequence from *E. coli* was amplified from plasmid pFaadAII (Koop et al., 1996) with the modifying primer 5'-TGAATTCCCATGGCTCGTGAAGCGG-3' (SEQ ID NO: 13) and 5'-GGTGATGATGATCCTTGCCAACTACCTTAGTGATCTC -3' (SEQ ID NO: 14) introducing a NcoI recognition site at the 5'-end. The PCR product was reamplified with primers 5'-TGAATTCCCATGGCTCGTGAAGCGG-3' (SEQ ID NO: 15) and 5'-GCTCTAGATTAGTGATGATGGTGATGATGATCCTTGCC-3' (SEQ ID NO: 16) to introduce a His-tag and XbaI recognition site at the 3'-end. The PCR product was digested with NcoI and XbaI. The pUC18 vector containing the right flanking region and the rpl32 promoter was digested with BamHI and XbaI and ligated with the digested psbA-5'-UTR and the digested aadA. The resulting plasmid was digested with XbaI and NdeI to remove the remaining pUC18 multicloning site. The digested plasmid was purified on an agarose gel. The band at 4600 bp was extracted purified and the ends filled in with Klenow polymerase. The plasmid was then religated, resulting in pICF742.

Please replace the paragraph that bridges pages 31 and 32 with the following paragraph:

The multicloning site of pUC18 between PaeI and SapI was removed and replaced by a new multicloning site consisting of (from 5' to 3') BamHI, KpnI, XbaI and NcoI. The left flanking region was amplified from tobacco plastid DNA (bp 131106 to bp 132277 of the *N. tabacum* plastome) with modifying primers 5'- GATGGATCCTTGCTGTTGCATCGAAAGAG -3' (SEQ ID NO: 17) and 5'- CACTGGTACCCGGGAATTGTGACCTCTCGGGAGAATC -3' (SEQ ID NO: 18), introducing a BamHI recognition site at the 5'-end and a KpnI recognition site at the 3'-end. The PCR product was purified and digested with BamHI and KpnI and ligated into the pUC18 plasmid with new multicloning site, which was digested with the same enzymes. The resulting plasmid was digested with KpnI and XbaI. The digested vector was ligated with the single strand oligonucleotide 5'-GATGTCTAGAAGCAACGTAAAAAACC CGCCCCGGCGGGTTTTT TATACCCGTAGTATCCCCAGCGGCCGCGGTAC-3' (SEQ ID NO: 19), coding for the E. coli alpha operon terminator. The complementary strand was filled in with Taq polymerase, digested with XbaI and religated. The resulting vector was digested with NcoI and XbaI and ligated with the aadA PCR product from pICF742, resulting in vector pICF743.

Please replace the paragraph that begins on page 32 at line 14 with the following paragraph:

Two spectinomycin/streptomycin resistant calli from cycle-0 were analysed by PCR to verify the transformation. Three different primer pairs were used (fig. 11):

- A) 5'-CAGACTAATACCAATCCAAGCC-3' (SEQ ID NO: 20) (binding outside the left flanking region at the *N. tabacum* plastome) and 5'-CTATCAGAGGTAGTTGGCGTC-3' (SEQ ID NO: 21) (binding at the marker gene).
- B) 5'-CACTACATTTTCGCTCATCGCC-3' (SEQ ID NO: 22) (binding at the marker gene) and 5'-TATTCCGACTTCCCCAGAGC-3' (SEQ ID NO: 23) (binding outside the right flanking region at the *N. tabacum* plastome)
- C) 5'-CATCAATACCTCGGTCTAG-3' (SEQ ID NO: 24) (binding at the left flanking region) and 5'-ACACATAGTATGCCCGGTC-3' (SEQ ID NO: 25) (binding at the right flanking region).

Please replace the paragraph that bridges pages 33 and 34 with the following paragraph:

The left flanking region was amplified from tobacco plastid DNA (complementary to bp 534 to bp 1336 of the *N. tabacum* plastome) with modifying primers 5'-TATAGGGCCCAGC TATAGGTTTACATTTTTTACCC-3' (SEQ ID NO: 26) and 5'-GTCCTGCAGTTATCCATTGTAGATGG AGCTTCG-3' (SEQ ID NO: 27), introducing a Bsp120I recognition site at the 5'-end and a PstI recognition site at the 3'-end. The PCR product was purified and digested with Bsp120I and PstI and ligated into the pICF5001 vector, which was digested with the same enzymes. Plasmid pICF5001 is a pUC18 derivative containing the modified multi cloning site 5'-GAATTCGGGCCCCGTCGACCCTGCAGGCCCGGGGATCCATATGCCATGGTCTAGATGATCATCATCACCA TCATCACTAATCTAGAGAGCTCCTCGAGGCGGCCGCGGTACCATGCATGCAAGCTT-3' (SEQ ID NO: 28). The ligation results in pICF5001 harbouring the left flanking region. The ribosomal binding site of gene 10 from phage T7 and an N-terminal fusion tag enhancing translation activity was introduced by inserting the synthetic nucleotide sequence 5'-CTGCAGGATCCTATA GGGAGACCACAACGGTTTCCCTCTAGTAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTA GCATTTCCATGG-3' (SEQ ID NO: 29) between the PstI and NcoI site of pICF5001 harbouring the left flanking region. The resulting vector was digested with NcoI and HindIII. The N-terminal fragment of *uidA* was amplified from *E. coli* DNA with modifying primers 5'-CATGCCATGGTCCGTCCTGTAGAA-3' (SEQ ID NO: 30) and 5'-GCCAAGCTTGTACAGTTCTTTCCG CTTGTTGCCC-3' (SEQ ID NO: 31), introducing a NcoI recognition site at the 5'-end and a HindIII recognition site at the 3'-end. The PCR product was purified and digested with NcoI and HindIII. The PCR product was then inserted into the vector, digested with the same enzymes, resulting in pICF1033.

Please replace the paragraph that begins on page 34 at line 12 with the following paragraph:

The ribosomal binding site of gene 10 from phage T7 was introduced into pICF5001 as described above. Modifying primers 5'-CATGCCATGGTCCGTCCTGTAGAA-3' (SEQ ID NO: 32) and 5'-CTGGGTACCTTATTGTTTGCCTCCCTGCTGCG-3' (SEQ ID NO: 33) were used to

amplify the complete *uidA* gene, while introducing a NcoI recognition site at the 5'-end and a KpnI recognition site at the 3'-end. The PCR product was digested with NcoI and KpnI and ligated into the vector containing the T7 ribosomal binding site, digested with the same enzymes. The *aadA* sequence from *E. coli* was amplified from plasmid pFaadAII (Koop et al., 1996) with the modifying primers 5'-GGATCCATGCGTGAAGCGTTATCGCCG-3' (SEQ ID NO: 34) and 5'-GGTGATGATGATCCTTGCCAACTACCTTAGTGATCTC-3' (SEQ ID NO: 35). The PCR product was reamplified with modifying primers 5'-GGGGTACCAGTTGTAGG-GAGGGATCCATGCGTGAAGC-3' (SEQ ID NO: 36) and 5'-GCTCTAGATTAGTGATGATGGTGATGATGATCCTTGCC-3' (SEQ ID NO: 37) to introduce a His-tag and XbaI recognition site at the 3'-end and a synthetic ribosomal binding site and KpnI recognition site at the 5'-end. The PCR product was purified and digested with KpnI and XbaI. The right flanking region was amplified from tobacco plastid DNA (complementary to bp 155370 to bp 533 of the *N. tabacum* plastome) with modifying primer 5'-CTAATCTAGAGAGCTCGTCTATAGGAGGTTTTGAAAAG-3' (SEQ ID NO: 38), introducing a XbaI recognition site at the 5'-end and exact primer 5'-CCAGAAAGAAGTATGCTTTGG-3' (SEQ ID NO: 39), binding behind a HindIII restriction site in the tobacco plastome. The PCR product was purified and digested with XbaI and HindIII. The vector containing the T7 ribosomal binding site and *uidA* gene was digested with KpnI and HindIII and then ligated with the two PCR products (digested with KpnI/XbaI resp. XbaI/HindIII), resulting in vector pICF1034.

Please replace the paragraph that begins on page 37 at line 6 with the following paragraph:

Spectinomycin resistant potato shoots were analysed by PCR to verify correct plastid transformation. Three different primer pairs were used as described for the analysis of tobacco transformants (example 2):

- A) 5'-CAGACTAATACCAATCCAAGCC-3' (SEQ ID NO: 20) (binding outside the left flanking region within the *S. tuberosum* plastome) and 5'-CTATCAGAGGTAGTTGGCGTC-3' (SEQ ID NO: 21) (binding within the *aadA* marker gene).
- B) 5'-CACTACATTTGCTCATCGCC-3' (SEQ ID NO: 22) (binding within the *aadA*

marker gene) and 5'-TATTCCGACTTCCCCAGAGC-3' (SEQ ID NO: 23) (binding outside the right flanking region within the *S. tuberosum* plastome)

- C) 5'-CATCAATACCTCGGTCTAG-3' (SEQ ID NO: 24) (binding within the left flanking region) and 5'-ACACATAGTATGCCCCGGTC-3' (SEQ ID NO: 25) (binding within the right flanking region).